ABSTRACT

Charles University
Faculty of Pharmacy in Hradec Králové
Department of Analytical Chemistry
Candidate: Denisa Dobrovolná
Supervisor: Doc. PharmDr. Hana Sklenářová, Ph.D.
Consultant: prof. Manuel Miró
Title of diploma thesis: Automation of determination using different types of luciferase

The aim of this diploma thesis was optimization the assay conditions by using sequential injection analysis. Two types of luciferase were used to optimize the method–type one and type two. Two different flow cells with different volume and optical path were used – the first flow cell with volume of 100 µl and 3x3 mm optical path and the second flow cell with volume of 120 µl and 5x5 mm optical path. The parameters of measurement were optimized – especially flow rate, accumulation time, cycle time and detector sensitivity. The method was first tested using an indirect luminal system to optimize fluorescence detector settings and reaction conditions. The best results were obtained with the flow cell with the volume of 120 µl and 5x5 mm optical path. Better measurement repeatability was observed using this flow cell. The measurement optimization continued using this flow cell by determining type one – non-secreted luciferase, and subsequently type two – secreted luciferase.

Due to unsatisfactory repeatability and distinct noise, the optimization will be subject to further testing, which will be done by using 3D print adapter to edit.